

AMENDMENTS TO THE SPECIFICATION

Amend paragraph [38] as follows:

[38] In a preferred embodiment, colorectal cancer sequences are those that are up-regulated in colorectal cancer; that is, the expression of these genes is higher in colorectal carcinoma as compared to normal colon tissue. "Up-regulation" as used herein means at least about 1.1 fold change, preferably a 1.5 or two fold change, preferably at least about a three fold change, with at least about five-fold or higher being preferred. All accession numbers herein are for the ~~GenBank~~ GENBANK® sequence database and the sequences of the accession numbers are hereby expressly incorporated by reference. ~~GenBank~~ The GENBANK® sequence database is known in the art, see, e.g., Benson, DA, et al., Nucleic Acids Research 26:1-7 (1998) and ~~<http://www.ncbi.nlm.nih.gov/>~~. In addition, these genes were found to be expressed in a limited amount or not at all in heart, brain, lung, liver, breast, kidney, prostate, small intestine and spleen.

Amend paragraph [45] as follows:

[45] The extracellular domains of transmembrane proteins are diverse; however, conserved motifs are found repeatedly among various extracellular domains. Conserved structure and/or functions have been ascribed to different extracellular motifs. For example, cytokine receptors are characterized by a cluster of cysteines and a WSXWS (SEQ ID NO: 3) (W=tryptophan, S=serine, X=any amino acid) motif. Immunoglobulin-like domains are highly conserved. Mucin-like domains may be involved in cell adhesion and leucine-rich repeats participate in protein-protein interactions.

Amend paragraph [54] as follows:

[54] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 70%, 75%, 80%, 85%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when

compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (~~see, e.g., NCBI web site <http://www.ncbi.nlm.nih.gov/BLAST/> or the like~~). Such sequences are then said to be "substantially identical." This definition also refers to, or may be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions, as well as naturally occurring, e.g., polymorphic or allelic variants, and man-made variants. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

Amend paragraph [57] as follows:

[57] Preferred examples of algorithms that are suitable for determining percent sequence identity and sequence similarity include the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, *Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al.*, *J. Mol. Bio.* 215:403-410 (1990). BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (~~<http://www.ncbi.nlm.nih.gov/>~~). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positivevalued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, e.g., for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0)

and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1992) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

Amend paragraph [188] as follows:

[188] Generally, in a preferred embodiment of the methods herein, the colorectal cancer protein or the candidate agent is non-diffusably bound to an insoluble support having isolated sample receiving areas (e.g. a microtiter plate, an array, etc). The insoluble supports may be made of any composition to which the compositions can be bound, is readily separated from soluble material, and is otherwise compatible with the overall method of screening. The surface of such supports may be solid or porous and of any convenient shape. Examples of suitable insoluble supports include microtiter plates, arrays, membranes and beads. These are typically made of glass, plastic (e.g., polystyrene), polysaccharides, nylon or nitrocellulose, ~~Teflon~~ TEFLON® non-stick coating, etc. Microtiter plates and arrays are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding of the composition is not crucial so long as it is compatible with the reagents and overall methods of the invention, maintains the activity of the composition and is nondiffusable. Preferred methods of binding include the use of antibodies (which do not sterically block either the ligand binding site or activation sequence when the protein is bound to the support), direct binding to “sticky”

or ionic supports, chemical crosslinking, the synthesis of the protein or agent on the surface, etc. Following binding of the protein or agent, excess unbound material is removed by washing. The sample receiving areas may then be blocked through incubation with ovine serum albumin (BSA), casein or other innocuous protein or other moiety.

Amend paragraph [237] as follows:

[237] Before using generator, it should have been cleaned after last usage by running it through soapy ~~H₂O~~ H₂O and using rinsing thoroughly. Run through with EtOH to sterilize. Keep tissue frozen until ready. Add ~~TRIzol~~ TRIZOL® reagent directly to frozen tissue then homogenize.

Amend paragraph [238] as follows:

[238] Following homogenization, remove insoluble material from the homogenate by centrifugation at 7500 x g for 15 min. in a ~~Servall~~ SORVALL® superspeed or 12,000 x g for 10 min. in an Eppendorf centrifuge at ~~4°C~~ 4°C. Transfer the cleared homogenate to a new tube(s). The samples may be frozen now at -60 to - ~~70°C~~ 70°C (and kept for at least one month) or you may continue with the purification.

Amend paragraph [242] as follows:

[242] Incubate samples at room temp. for 2-3 minutes. Centrifuge samples at 6500rpm in a ~~Servall~~ SORVALL® superspeed for 30 min. at ~~40°C~~ 40°C. (You may spin at up to 12,000 x g for 10 min. but you risk breaking your tubes in the centrifuge.)

Amend paragraph [243] as follows:

[243] Transfer the aqueous phase to a fresh tube. Save the organic phase if isolation of DNA or protein id desired. Add 0.5ml of isopropyl alcohol per 1ml of ~~TRIzol~~ TRIZOL® reagent used in the original homogenization. Cap tubes securely and

invert to mix. Incubate samples at room temp. for 10 minutes. Centrifuge samples at 6500rpm in ~~Servall~~ a SORVALL® centrifuge for 20 min. at ~~40°C~~ 40°C.

Amend paragraph [244] as follows:

[244] Pour off the supernate. Wash pellet with cold 75% ethanol. Use 1ml of 75% ethanol per 1ml of ~~TRIZOL~~ TRIZOL® reagent used in the initial homogenization. Cap tubes securely and invert several times to loosen pellet. (do not vortex). Centrifuge at <8000rpm (<7500 x g) for 5 minutes at ~~4°C~~ 4°C.

Amend paragraph [254] as follows:

[254] Add 0.4 vol. of 7.5 M NH_4OAc + 2.5 vol. of cold 100% ethanol. Precipitate at ~~-20°C~~ 20°C 1 hour to overnight (or 20-30 at ~~-70°C~~ 70°C). Centrifuge at 14,000-16,000 x g for 30 minutes at ~~4°C~~ 4°C. Wash pellet with 0.5 ml of 80% ethanol (~~-20°C~~ 20°C) then centrifuge at 14,000-16,000 x g for 5 minutes at room temperature. Repeat 80% ethanol wash. Dry the last bit of ethanol from the pellet in the hood. (Do not speed vacuum). Suspend pellet in DEPC ~~H₂O~~ H₂O at 1 ~~µg/µl~~ µg/µl concentration.

Amend paragraph [255] as follows:

[255] Add no more than 100 ~~µg~~ µg to an RNeasy column. Adjust sample to a volume of 100 ~~µl~~ µl with RNase-free water. Add 350ul Buffer RLT then 250 ~~µl~~ µl ethanol (100%) to the sample. Mix by pipetting (do not centrifuge) then apply sample to an RNeasy mini spin column. Centrifuge for 15 sec at >10,000 rpm. If concerned about yield, re-apply flowthrough to column and centrifuge again.

Amend paragraph [256] as follows:

[256] Transfer column to a new 2-ml collection tube. Add 500 ~~µl~~ µl Buffer RPE and centrifuge for 15 sec at >10,000rpm. Discard flowthrough. Add 500 ~~µl~~ µl Buffer RPE and centrifuge for 15 sec at >10,000rpm. Discard flowthrough then centrifuge for 2

min at maximum speed to dry column membrane. Transfer column to a new 1.5-ml collection tube and apply 30-50 μ l of RNase-free water directly onto column membrane. Centrifuge 1 min at >10,000rpm. Repeat elution.

Amend paragraph [259] as follows:

[259] Use 5 μ g of total RNA or 1 μ g of polyA+mRNA as starting material. For total RNA, use 2 μ l of SuperScript RT. For polyA+mRNA, use 1 μ l of SuperScript RT. Final volume of first strand synthesis mix is 20 μ l. RNA must be in a volume no greater than 10 μ l. Incubate RNA with 1 μ l of 100pmol T7-T24 oligo for 10 min at 70°C. On ice, add 7 μ l of: 4 μ l 5X 1st. Strand Buffer, 2 μ l of 0.1M DTT, and 1 μ l of 10mM dNTP mix. Incubate at 37°C for 2 min then add SuperScript RT

Incubate at 37°C for 1 hour.

Second Strand Synthesis

Place 1st strand reactions on ice.

Add: 91 μ l DEPC H2O

30 μ l 5X 2nd Strand Buffer

3 μ l 10mM dNTP mix

1 μ l 10U/ μ l E.coli DNA Ligase

4 μ l 10U/ μ l E.coli DNA Polymerase

1 μ l 2U/ μ l RNase H

Amend paragraph [260] as follows:

[260] Make the above into a mix if there are more than 2 samples. Mix and incubate 2 hours at 16°C.

Amend paragraph [261] as follows:

[261] Add 2 ~~ml~~ μl T4 DNA Polymerase. Incubate 5 min at 16°C. Add 10 ~~ml~~ μl of 0.5M EDTA

Amend paragraph [264] as follows:

[264] Centrifuge PLG tubes for 30 sec at maximum speed. Transfer cDNA mix to PLG tube. Add equal volume of phenol-chloroform:isamyl alcohol and shake vigorously (do not vortex). Centrifuge 5 minutes at maximum speed. Transfer top aqueous solution to a new tube. Ethanol precipitate: add 7.5X 5M NH₄OAc and 2.5X volume of 100% ethanol. Centrifuge immediately at room temp. for 20 min, maximum speed. Remove sup then wash pellet 2X with cold 80% ethanol. Remove as much ethanol wash as possible then let pellet air dry. Resuspend pellet in 3 ~~ml~~ μl RNase-free water.

In vitro Transcription (IVT) and labeling with biotin

Pipet 1.5 ~~ml~~ μl of cDNA into a thin-wall PCR tube.

Make NTP labeling mix:

Combine at room temperature: 2 ~~ml~~ μl T7 10xATP (75mM) (Ambion)

2 ~~ml~~ μl T7 10xGTP (75mM) (Ambion)

1.5 ~~ml~~ μl T7 10xCTP (75mM) (Ambion)

1.5 ~~ml~~ μl T7 10xUTP (75mM) (Ambion)

3.75 ~~ml~~ μl 10mM Bio-11-UTP (Boehringer-Mannheim/Roche or Enzo)

3.75 ~~ml~~ μl 10mM Bio-16-CTP (Enzo)

2 ~~ml~~ μl 10xT7 transcription buffer (Ambion)

2 ~~ml~~ μl 10xT7 enzyme mix (Ambion)

Amend paragraph [265] as follows:

[265] Final volume of total reaction is 20 ~~ml~~ μl. Incubate 6 hours at 37°C in a PCR machine.

Amend paragraph [268] as follows:

[268] 15 ~~ug~~ μg of labeled RNA is usually fragmented. Try to minimize the fragmentation reaction volume; a 10 ~~ul~~ μl volume is recommended but 20 ~~ul~~ μl is all right. Do not go higher than 20 ~~ul~~ μl because the magnesium in the fragmentation buffer contributes to precipitation in the hybridization buffer.

Amend paragraph [269] as follows:

[269] Fragment RNA by incubation at 94 °C for 35 minutes in 1 x Fragmentation buffer.

5 x Fragmentation buffer:

200 mM Tris-acetate, pH 8.1

500 mM KOAc

150 mM MgOAc

Amend paragraph [270] as follows:

[270] The label RNA transcript can be analyzed before and after fragmentation. Samples can be heated to 65°C for 15 minutes and electrophoresed on 1% agarose/TBE gels to get an approximate idea of the transcript size range.

Amend paragraph [271] as follows:

[271] 200 ~~ul~~ μl (10 ~~ug~~ μg cRNA) of a hybridization mix is put on the chip. If multiple hybridizations are to be done (such as cycling through a 5 chip set), then it is recommended that an initial hybridization mix of 300 ~~ul~~ μl or more be made.

Hybridization Mix: fragment labeled RNA (50mg/~~ul~~ μl final conc.)

50 pM 948-b control oligo

1.5 pM BioB

5 pM BioC

25 pM BioD
100 pM CRE
0.1mg/ml herring sperm DNA
0.5mg/ml acetylated BSA
to 300 ~~ul~~ ul with 1xMES hyb. buffer

Amend paragraph [272] as follows:

[272] The instruction manuals for the products used herein are incorporated herein in their entirety.

Labeling Protocol Provided Herein

Hybridization reaction:

Start with non-biotinylated IVT (purified by RNeasy columns
(see example 1 for steps from tissue to IVT)

IVT antisense RNA; 4 μ g: μ l

Random Hexamers (1 μ g/ μ l): 4 μ l

H₂O H₂O: μ l

14 μ l

- Incubate 70°C, 10 min. Put on ice.

Reverse transcription:

5X First Strand (BRL) buffer: 6 μ l

0.1 MDTT: 3 μ l

50X dNTP mix: 0.6 μ l

H₂O H₂O: 2.4 μ l

Cy3 or Cy5 dump (1mM): 3 μ l

SS RT II (BRL): 1 μ l

16 μ l

- Add to hybridization reaction.
- Incubate 30 min., 42°C.
- Add 1 µl SSII and let go for another hour.

Put on ice.

- 50X dNTP mix (25mM of cold dATP, dCTP, and dGTP, 10mM of dTTP: 25 µl each of 100mM dATP, dCTP, and dGTP; 10 µl of 100mM dTTP to 15 µl H_2O H_2O . dNTPs from Pharmacia)

RNA degradation:

86 µl H_2O H_2O

- Add 1.5 µl 1M NaOH/2mM EDTA, incubate at 65°C, 10 min.

10 µl 10N NaOH

4 µl 50mM EDTA

U-Con 30

500 µl TE/sample spin at 7000g for 10 min, save flow through for purification

Qiagen QIAGEN® purification:

- suspend u-con recovered material in 500µl buffer PB
- proceed w/normal Qiagen QIAGEN® protocol

DNase digest:

- Add 1 µl of 1/100 dil of DNase/30µl Rx and incubate at 37°C for 15 min.
- 5 min 95°C to denature enzyme

Sample preparation:

- Add:

Cot-1 DNA: 10 µl

50X dNTPs: 1 µl

Na pyro phosphate: 7.5 µl

10mg/ml Herring sperm DNA 1 µl of 1/10 dilution

21.8 final vol.

- Dry down in speed vac.

- Resuspend in 15 μ l H_2O
- Add 0.38 μ l 10% SDS.
- Heat 95°C, 2 min.
- Slow cool at room temp for 20 min.

Put on slide and hybridize overnight at 64°C.

Washing after the hybridization:

3X SSC/0.03% SDS: 2 min. 37.5 ml 20X SSC+0.75ml 10% SDS in
250ml H_2O

| | | |
|-----------|--------|---|
| 1X SSC: | 5 min. | 12.5 ml 20X SSC in 250ml H_2O |
| 0.2X SSC: | 5 min. | 2.5 ml 20X SSC in 250ml H_2O |

Dry slides in centrifuge, 1000 RPM, 1 min.

Amend the paragraph beginning on page 109, line 1 as follows:

Table 2 provides the nucleic acid (SEQ ID NO: 1) and protein sequence (SEQ ID NO: 2) of the CBF9 gene as well as the Unigen and Exemplar accession numbers for CBF9.